Data Evaluation Record on the Acute Toxicity of AE F159481 Technical (metabolite of Glufosinate Ammonium) to Aquatic Vascular Plants, *Lemna gibba*

EPA MRID Number 48444815

Data Requirement:	EPA DP Barcode	345709
	EPA MRID	48444815
	EPA Guideline	850.4400

Test material: **AE F159481 Technical (metabolite of Glufosinate Ammonium)** **Purity:** 98.9% w/w
Common name
Chemical name: IUPAC 2-methylphosphinico-acetic acid
CAS name
CAS No.
Synonyms

Primary Reviewer: Moncie Wright **Signature:** Moncie V Wright
Staff Scientist, Cambridge Environmental Inc. **Date:** 7/26/11

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, Cambridge Environmental Inc. **Date:** 10/19/11

Primary Reviewer: Catherine Aubee **Signature:**
Biologist, US EPA/OPP/EFED/ERBIV **Date:** 1 June 2012

EPA PC Code 128850

Date Evaluation Completed: 01-06-2012

CITATION: Sowig, P. and O. Weller. 2001. Duckweed (*Lemna gibba* G3) Growth Inhibition Test – AE F159481, substance, technical (Metabolite of AE F039866). Unpublished study performed and sponsored by Aventis CropScience GmbH, Frankfurt am Main, Germany. Study completed June 5, 2001.

EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, the freshwater floating aquatic vascular plants duckweed, *Lemna gibba*, were exposed to **AE F159481 Technical** under static conditions at nominal concentrations of 0 (negative control), 10, 18, 32, 56, and 100 mg ai /L, which were equivalent to 0 (negative control), 9.89, 17.8, 31.65, 55.38, and 98.9 mg ai/L (adjusted for % purity). Mean-measured concentrations were <LOQ (<0.64, control), 9.61, 17.0, 31.3, 53.5, and 97.2 mg ai/L.

The % growth inhibition of frond number in the treated culture as compared to the control ranged from -1 to 1%. The most sensitive endpoint could not be determined as there was no toxicity in this study, resulting in overall NOAEC and EC_{50} values of 97.2 and >97.2 mg ai/L, respectively.

There were no compound related phytotoxic effects.

This toxicity study is scientifically sound is classified as **acceptable**. It is consistent with the guideline for a Tier II aquatic plant toxicity study with *Lemna gibba* exposed to a glufosinate transformation product (MPA).

Results Synopsis

Test Organism: *Lemna gibba*
Test Type (Flow-through, Static, Static Renewal): Static renewal

Frond number, growth rate, and biomass increase
EC_{05}: >97.2 mg ai/L 95% C.I.: N/A
EC_{50}: >97.2 mg ai/L 95% C.I.: N/A
NOAEC: 97.2 mg ai/L
Probit Slope: N/A
Endpoint(s) Effected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted according to OECD draft guideline of June 1998: *Lemna*, Growth Inhibition Test; U.S. EPA Pesticide Assessment Guidelines Subdivision J, Hazard Evaluation: Nontarget Plants §123-2: Growth and reproduction of aquatic plants (Tier 2; 1982); U.S. EPA OPPTS 850.4400: Aquatic plant toxicity test using *Lemna* spp., Tiers I and II; and ASTM E 1415-91: Standard Guide for Conducting Static Toxicity Tests with *Lemna gibba* G3 (1991). The study methods and results were assessed according to U.S. EPA OPPTS 850.4400 and OECD Guideline No. 221:, and differences and or similarities were described. A deficiency and deviations were noted:

1. The total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water were not determined. Lack of such information can render a study invalid. However, the control and treatment plants displayed no phytotoxic effects and did not experience significant inhibitions in the endpoints tested.
2. The study author did not report the age of the inocula; OPPTS guidelines suggest that inocula be taken from cultures which are less than 2 weeks old. OECD guidelines suggest that cultures be 7-10 days old.
3. The physico-chemical properties of the test material were not reported.
4. The pre-test health of the plants was not reported.
5. The pH of the control and test solutions ranged from 7.5 to 9.0; OPPTS guidelines suggest that pH be 7.5±0.1. However, OECD guidance only suggests that pH not drift by more than 1.5 units in the control solutions.

The deficiency and deviationsdo not have a substantive impact on the acceptability of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted in compliance with the Principles of Good Laboratory Practice as adopted by the council of OECD on 26th November, 1997 [C(97)186/(Final)] for implementation at the national level.

A. MATERIALS:

1. Test material **AE F159481 Technical (metabolite of Glufosinate Ammonium)**

Description: White powder

Lot No./Batch No. : Not reported

Purity: 98.9% w/w

Stability of compound under test conditions: Analytical verification performed on fresh solutions for days 0, 3, and 5 yielded recoveries ranging from 96 to 109% of the nominal test concentrations. The spent solutions from days 3, 5, and 7 had recoveries ranging from 87 to 105% of the nominal test concentrations. The test material was stable under the test conditions.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test

compound)

Storage conditions of test chemicals: Not reported.

Physicochemical properties of AE F159481 Technical.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Name: Duckweed, *Lemna gibba* *EPA requires a vascular species: Lemna gibba.*
Strain, if provided: G3
Source: In-house cultures were originally obtained from the Plant Hormone Laboratory, United States Department of Agriculture, Beltsville, Maryland.
Age of inoculum: 6 weeks
Method of cultivation: Plants were cultured in 20X-AAP nutrient medium.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study A range-finding study was not conducted.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	6 weeks Same as test (temperature and medium). Not reported	
Test system		

Parameter	Details	Remarks ***Criteria***
Static/static renewal Renewal rate for static renewal	Static renewal Days 3 and 5	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	The test vessels were placed in a waterbath; the incubation facility was not reported.	
Duration of the test	7 days	*EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
<u>Test vessel</u> Material: *(glass/stainless steel)* Size: Fill volume:	 Glass 300 mL 150 mL	

Parameter	Details	Remarks *Criteria*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source:	 7.5 9.0 Yes $NaHCO_3$	*EPA recommends the following culture media: Modified Hoagland's E+ or 20X-AAP. Chelating agents (e.g. EDTA) are recommended in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Standard medium was used.	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Deionized water used to create reagent grade water; source not reported 7.5 after adjustment with HCl Filtered by ultrafiltration, ion exchange, and a charcoal unit. Not reported. Not reported. Not reported. Not reported. Not reported.	*EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*
Indicate how the test material is added to the medium (added directly or used stock solution)	The test substance (0.1 mg) was dissolved in the dilution water to prepare a primary stock solution. Defined amounts of the stock solution well shaken before extraction were pipetted proportionally into the flasks filled with nutrient medium.	

Parameter	Details	Remarks *Criteria*
Aeration or agitation	Neither	
Sediment used (**for rooted aquatic vascular plants**) Origin: Textural classification (%sand, silt, and clay): Organic carbon (%): Geographic location:	N/A	
Number of replicates Control: Solvent control: Treatments:	 3 N/A 3	
Number of plants/replicate	3-4 plants per replicate	*EPA requires 5 plants.*
Number of fronds/plant	3-4 fronds per plant	*EPA requires 3 fronds per plant.*
Test concentrations Nominal (not adjusted for purity): Nominal (adjusted for % purity of the test material): Mean-Measured:	 0 (negative control), 10, 18, 32, 56, and 100 mg ai/L 0 (negative control), 9.89, 17.80, 31.65, 55.38, and 98.9 mg ai/L <LOQ (<0.64, control), 9.61, 17.0, 31.3, 53.5, and 97.2 mg ai/L	*EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	N/A- no solvent was used	
Method and interval of analytical verification	Samples from the control and all treatment levels were taken from all freshly prepared solutions (days 0, 3, and 5) and all spent solutions (days 3, 5, and 7) and analyzed via HPLC with UV detection (216 nm).	Fortification and method validation samples and matrix and solvent blanks were analyzed concurrently.

Parameter	Details	Remarks *Criteria*
Test conditions Temperature: Photoperiod: Light intensity and quality:	 24.5-25.0°C Continuous Wide spectrum fluorescent lamps of universal white 59.1-60.4 µE*m^{-2}*s^{-1}	
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (e.g.,: number of fronds, plant dry weight or other toxicity symptoms)	- Frond number - Growth rate (based on frond number) - Dry weight increase	
Measurement technique for frond number and other end points	The method of determination for frond number was not reported. Growth rate was determined using a logarithmic equation that accounts for the average number of fronds observed at the beginning and end of the test. The increase in biomass was determined by subtracting initial biomass from final biomass.	
Observation intervals	Days 3, 5, and 7	
Other observations, if any	None	
Indicate whether there was an exponential growth in the control	Yes; frond number was 175 fronds/mL at test termination.	
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Frond number averaged 175 fronds/mL in the negative control, yielding reviewer-calculated inhibitions of 0, -1, -1, -1, and 1% in the nominal 10, 18, 32, 56, and 100 mg ai/L (unadjusted for purity) test levels, respectively. The study author did not analyze this endpoint.

Growth rate in the negative control averaged 0.383 days-1, yielding inhibitions of 0, 0, 0, 0, and 0%.

The mean increase in biomass in the negative control was 16.1 mg, yielding inhibitions of 1, 2, 0, 2, and 0%.

No phytotoxic effects were observed.

Table 3: Effect of AE F159481Technical on frond number of Duckweed, *Lemna gibba*.

Treatment Mean-measured (and nominal) concentrations (mg ai/L)	Initial frond number/test solution	frond number at			
		3 days	5 days	7 days	
				frond number	% inhibition
Negative control	12	32	84.3	175	N/A
9.61 (9.89)	12	32	82	175	0
17.0 (17.80)	12	31.3	86.3	176	-1
31.3 (31.65)	12	32.3	82.7	176	-1
53.5 (55.38)	12	33	83.3	177	-1
97.2 (98.9)	12	32.3	84.3	174	1
Reference chemical (if used)	N/A				

Table 5: Effect of AE F159481Technical on growth of Duckweed, *Lemna gibba*.

Mean-Measured and (Nominal) Concentrations mg ai/L	Initial frond number/test solution	Growth rate based on frond number (days $^{-1}$, mean)	Growth rate % Inhibition	Biomass increase (mg)	Biomass % Inhibition
Negative control	12	0.383	N/A	16.1	N/A
9.61 (9.89)	12	0.383	0	15.8	1
17.0 (17.80)	12	0.384	0	15.7	2
31.3 (31.65)	12	0.383	0	16.0	0
53.5 (55.38)	12	0.384	0	15.7	2
97.2 (98.9)	12	0.382	0	16.0	0
Reference chemical (if used)	N/A				

Table 5: Statistical endpoint values.*

Statistical Endpoint	Frond No.	Growth Rate (based on frond no.)	Biomass Increase
NOAEC or EC_{05} (mg technical concentrate/L)	ND	100	100
EC_{50} (mg technical concentrate/L)	ND	>100	>100
Reference chemical NOAEC IC_{50}/EC_{50}	N/A		

* Toxicity values based on nominal concentrations unadjusted for % purity.

B. REPORTED STATISTICS:

The NOEC was determined using ANOVA and General Linear Models with Duncan's Multiple Range Test Procedures using SAS (1989). The nominal concentrations that were unadjusted for % purity were used for analysis.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: There were inhibitions of <5% for frond number, growth rate, and biomass increase. The reviewer visually determined all toxicity values using the mean-measured concentrations.

Frond number, growth rate, and biomass increase
EC_{05}: >97.2 mg ai/L 95% C.I.: N/A
EC_{50}: >97.2 mg ai/L 95% C.I.: N/A
NOAEC: 97.2 mg ai/L
Probit Slope: N/A

D. STUDY DEFICIENCIES:

The total organic carbon, particulate matter, metals, pesticides, and chlorine content of the dilution water were not determined.

E. REVIEWER'S COMMENTS:

The reviewer's and the study author's results were in complete agreement; there was no toxicity in this study. However, the reviewer included frond number in the analysis and used mean-measured concentrations; therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

The experiment was initiated April 9, 1999 and was terminated April 16, 1999.

F. CONCLUSIONS:

This toxicity study is scientifically sound is classified as **acceptable**. It is consistent with the guideline for a Tier II aquatic plant toxicity study with *Lemna gibba* exposed to a glufosinate transformation product (MPA). The most sensitive endpoint could not be determined as there was no toxicity in this study, resulting in overall NOAEC and EC_{50} values of 97.2 and >97.2 mg ai/L, respectively.

Frond number, growth rate, and biomass increase
EC_{05}: >97.2 mg ai/L 95% C.I.: N/A
EC_{50}: >97.2 mg ai/L 95% C.I.: N/A
NOAEC: 97.2 mg ai/L
Probit Slope: N/A
Endpoint(s) Effected: None

III. REFERENCES:

Organization for Economic Cooperation and Development, Draft OECD Guideline for Testing of Chemicals Guideline: *Lemna*, Growth Inhibition Test, April 1997.

U.S. Environmental Protection Agency (EPA), 1982. Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants; Tier 2 of Non-target Area Testing; §123-2 Growth and reproduction of aquatic plants.

U.S. Environmental Protection Agency (EPA), April 1996, Ecological Effects Test Guidelines: OPPTS 850.4400: Aquatic Plant Toxicity Test Using Lemna spp., Tiers I and II; EPA 712-C-96-156; Public Draft.

ASTM (1991). Standard Guide for Conducting Static Toxicity Tests with *Lemna gibba* G3. American Society for Testing and Materials. E 1415-91.

U.S. Environmental Protection Agency (EPA), 1983. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR Part 792). Fed. Reg.,Vol. 48, No. 230, Nov. 23, 1983, pp. 53922-53944.

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